

SEXUAL AND ASEXUAL PROPAGATION OF RED ELM (*ULMUS RUBRA*), GREY
ALDER (*ALNUS INCANA*), AND BUFFALOBERRY (*SHEPHERDIA CANADENSIS*)

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BRENDA MICHELLE MORALES RIVERA

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Approved by;

Co-Major Professor
Dr. Cheryl Boyer

Approved by;

Co-Major Professor
Dr. Charles Barden

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Abstract

Red elm (*Ulmus rubra*), grey alder [*Alnus incana* and *A. incana* spp. *tenuifolia*] and buffaloberry (*Shepherdia canadensis*) are considered important plants for many Native American tribes in the United States. Native Americans have used these three species for a variety of medicinal uses and ceremonial purposes. Currently, Kansas tribal leaders would like to plant more of these species on tribal land, but the plants have been difficult to propagate. While red elm is valued as a ceremonial tree, it is susceptible to Dutch Elm Disease caused by the fungus (*Ophiostoma ulmi*) and is not grown in many ornamental nurseries. This has led to declining tree populations. The objective of these studies were to evaluate methods to propagate red elm, grey alder and buffaloberry in order to find techniques that can lead to an increase in the production of these species commercially and enable tribes and landowners to increase the presence of these native plants on their lands. In the first study, the use of gibberellic acid (GA₃) was investigated to determine the optimum concentration needed for maximum seed germination. Studies were conducted with stratified (cold, moist storage) and non-stratified red elm, grey alder, and buffaloberry seeds soaked in one of four treatments: 0, 250, 500 or 1000 ppm of GA₃ in 2010, and 0, 500, 1000, 2000 ppm of GA₃ in 2011. Results indicate the use of GA₃ in high concentrations promoted germination of unstratified seeds of red elm, though low seed viability in grey alder and buffaloberry resulted in poor germination. In a second study, vegetative cuttings were treated with potassium indole-3-butyric acid (K-IBA) and Dip 'N GrowTM containing indole-3-butyric acid and naphthalene acetic acid (IBA + NAA). Softwood cuttings for the three species were treated with K-IBA and Dip 'N GrowTM (plant rooting hormones): K-IBA at 5,000 and 10,000 ppm and Dip 'N GrowTM at 1:10 ratio solutions. Results of this study showed that only grey alder softwood cuttings had callus formation, root growth, and shoot growth while red elm and buffaloberry did not respond to cutting propagation treatments.

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Dedication

To Mariana Elvir, Brenda Maritza Rivera and Jorge Morales.

Chapter 1 - Literature Review

Introduction

Native Americans made use of many plants for medicine, food, dye, and fibers. According to literature about Native American ethnobotany (Moerman, 1998), 2,582 plant species in the United States have been reported to have medicinal properties. Plants produce chemicals which can be used for different purposes such as treating skin problems, as an analgesic, or for diuretic remedies. Some plants can also be used to make baskets, thread, cords, and mats. Overall, there are an estimated 1,649 native plant species that were reportedly used for food, 442 for fiber, and 217 for dye (Moerman, 1998).

Natural populations of plants like red elm (*Ulmus rubra* Muhl.), grey alder (*Alnus incana*, (L.) Moench), and buffaloberry (*Shepherdia canadensis*, Nutt.) are in decline in many regions. These species have little commercial value and therefore receive little attention from nursery growers and plant breeders. Red elm in particular, shows little incentive for commercial ventures, because it has low potential monetary return due to its susceptibility to Dutch Elm Disease caused by the fungus (*Ophiostoma ulmi*). However, these plants are part of a natural and cultural heritage. Furthermore, they may serve an important role in maintaining the integrity of ecosystems and sustaining wildlife in their native ranges.

Red Elm (*Ulmus rubra*)

Red elm (*Ulmus rubra* Muhl.), also called slippery elm, is considered an important tree species for many Native American tribes. It is a deciduous tree which has reddish-brown colored heart wood that can reach from 40 - 60 ft in height (Fig. 1.1). It has alternate, simple, oblong leaves, from 3-7 in long, with a rough texture on the upper leaf surface. Lateral buds are

approximately 0.25 in long on a stem grayish-brown in color with mucilaginous characteristics. The fruit is an ovate wafer 0.25 - 0.75 in long, and covered with red-brown hairs. Seeds are small and covered with a wing (Anderson, 2010). According to Cooley (1990), red elm extends from southwestern Maine west to New York, extreme southern Quebec, southern Ontario, northern Michigan, central Minnesota, and eastern North Dakota; south to eastern South Dakota, central Nebraska, eastern Oklahoma, and central Texas; then east to northwestern Florida and Georgia (Fig. 1.2). Reported flowering dates are from February to May, with fruit ripening and dispersal dates are from April to June (Anderson, 2010).

Red elm has many uses in traditional medicine. The Potawatomi tribe used the boiled inner bark as a dermatological aid for inflammation (Erichsen-Brown, 1989). Eye medicine and throat aid were made from the inner bark (Moerman, 1998). Additionally, Native Americans used the bark for cramps and diarrhea (Erichsen-Brown, 1989). Bark and macerated leaves were used to treat colds and coughs. Women would drink a tea of the bark to make childbirth easier (Anderson, 2010). Each tribe had their own uses for medicinal plants. The Pawnee, Dakota, and Ponca tribes use the inner bark to make a laxative decoction. Currently, red elm bark is used as an ingredient in throat comfort teas which are sold commercially (Yogi Throat comfort, Golden Temple of Oregon, Springfield, Oregon) (Fig.1.3).

Red elm was also used as food and fiber. The Kiowa tribe would store the inner bark and use it to brew a tea during the winter. It could also be used for flavoring. The Potawatomi tribe used the bark to make boxes, baskets, ropes and cords (Moerman, 1998).

North American Indian tribes still use logs of red elm for ceremonial fires at pow wows (gathering of North American people), funerals and sweat lodges. It is still the preferred species for various ceremonial fires. Its wood is more dense than that of American elm (*Ulmus*

americana L.) thus, when burned, it provides more heat (USDA Forest Service, 2010). However, both species of elm are susceptible to Dutch elm disease, which is caused by a fungus (*Ophiostoma ulmi*) that affects the vascular tissue and prevents water movement to the crown. Eventually the tree will wilt and die, thus they are not widely grown in the nursery trade (Hutchinson, 1992).

Red elm seeds should be harvested when they are pale olive in color, between the months of April and June. Seeds should be air dried for several days, to improve storability yet too much air drying may reduce viability. They may need stratification (the process of pre-treating seeds to simulate natural winter conditions), which weakens the seed covering to allow the embryo to emerge from the seed coat. Stratification of red elm for 60-90 days with moist media is recommended due to the hardness of the seed coat (USDA, 2011).

Grey Alder (*Alnus incana*, *Alnus incana* ssp. *tenuifolia*)

Grey alder [*Alnus incana*, (L.) Moench] also known as tag alder, mountain alder, or hazel alder, is a large pyramidal to oval-crowned tree that reaches 40 to 60 ft in height. It has alternate, simple, ovate leaves, 2 to 4 in long which are dark green in color (Dirr, 2009), and has a reddish-brown, thin, smooth bark (Fig. 1.4). Grey alder is a species that grows in moist lowlands, (Fig. 1.5) common in regions surrounding the Great Lakes, and extending to east-central Canada, Virginia and Maryland (USDA, 2011), and is therefore well adapted to areas susceptible to periodic flooding. It is typically found bordering streams, rivers and mountain springs. Grey alder seeds are collected in September and October and can be stored for many years (USDA, 2011).

Sub-species of grey alder, *Alnus incana* ssp. *tenuifolia* (Nutt.), also known as thin leaf alder, has similar characteristics to *Alnus incana* except that it is considered a shrub or small tree,

20 -30 ft in height that grows throughout the mountain ranges of western North America (Jones, et.al., 2002) (Fig. 1.5). Seeds exhibit dormancy which can be broken by cold stratification (USDA, 2011).

Grey alder was used as a dermatological aid and as an eye medicine. Iroquois tribes used it as an anti-hemorrhagic compound, and Okanagan-Colville tribes used it as dietary aid infusion (Moerman, 1998). Additionally, fresh leaves can be used to make an astringent to treat tumors and inflamed tissues (Erichsen-Brown, 1989). Native Americans used grey alder to make a beverage to control fever. Twigs of the grey alder are steeped and used for bathing purposes, for backaches as well as headaches. An infusion of the inner bark can be used to reduce swellings. The Potawatomi tribe scraped the inner bark to obtain juice which could be used to treat inflammation (USDA, 2011). Grey alder was also used as a dye to restore shoes to brown and other color combinations.

Additionally, grey alder supports symbiotic nitrogen (N)-fixing bacteria in root nodules, which makes it valuable for improving soil fertility (USDA, 2011). Grey alder is an actinorhizal N₂-fixing tree species that can effectively achieve biological fertilization of the soil with nitrogen (Granhall, 1994). Alder species have a positive impact on the diversity and activity of soil microbial communities and it has been reported to increase soil phosphorus availability (Giardina et al., 1995).

Grey alder seeds should be collected in September or October and stored at room temperature. Stratification may be needed for a period of 60-90 days. However, past research has reported poor germination (USDA, 2011).

Buffaloberry (*Shepherdia canadensis*)

Buffaloberry (*Shepherdia canadensis* Nutt.) is also known as soapberry, russet red buffaloberry, or Canadian buffaloberry (Dirr, 2009). It is a perennial, nitrogen-fixing shrub that is well adapted to alkaline, saline soils and is drought resistant (USDA, 2011). Buffaloberry can reach 6 - 8 ft in height, and grows in USDA Hardiness Zones 2 - 6. It has leaves that are opposite, simple, ovate, and 0.5 - 2 in long, silver-green to gray-green in color, (Fig.1.6) and fruits are yellowish-red, and approximately 0.25 in in size (Moerman, 1998). According to USDA (2011), the shrub grows from Alaska to Maine, South Dakota, and the mountains of Arizona (Fig. 1.7).

Fresh berries of buffaloberry were used to treat gastro-intestinal problems, and a decoction of stems was used to control constipation. Berry juice was used to prevent heart attacks and indigestion. The berries were also chewed to induce childbirth, and the fruits can be eaten fresh or dried as well as for making “Indian ice cream” (USDA, 2011).

Buffaloberry seeds exhibit multiple dormancies primarily due to its hard seed coat (Rosner and Harrington, 2003). According to Rosner and Harrington (2003), acid scarification, cold moist stratification or both treatments are recommended for seed propagation of buffaloberry. They recommended acid scarification for a period between 15 to 30 minutes is required, followed by a stratification period.

Seed Germination

Seeds are divided into three important parts: embryo, food storage tissue, and seed covering (Hartmann and Kester, 2011). Fertilization of female by male gametes results in the embryo. Storage tissues are usually in the endosperm with the cotyledons digested by the

embryo. Seed coverings are the seed coats (outer protective covering of the seed), and are formed by the integuments of the ovule (Hartmann and Kester, 2011).

Seed germination is the emergence and development from the seed embryo and the ability to produce a normal plant under favorable conditions (Copeland, 1976). It can also be defined as the rupture of the seed coat followed by an active growth of the embryo which will result in a new plant. This process includes enzyme activation, initiation of the embryo growth, rupture of the seed coat, and emergence of the seedling (Copeland, 1976).

In the process of seed germination, water imbibition takes place. The cells within the seed become turgid and permeable to allow the exchange of oxygen and carbon dioxide with the atmosphere. Water absorbed by the seeds induces the activation of enzymes, the embryo starts to develop, a radicle emerges, and cell elongation begins (Copeland, 1976). When green tissue develops, the seedling is able to photosynthesize and produce its own carbohydrates. There are several factors that may affect seed germination such as: seed maturity, viability, as well as several environmental factors specific to water, gaseous exchange, and temperature (optimum germination temperature of many species is between 59 °F and 86 °C (Copeland, 1976).

Seed Viability

A viable seed is a seed that has the potential to germinate in the soil and adapt to different conditions (Aslam et al., 2010). A common method to test seed viability is the tetrazolium test. This method was developed in Germany by Georg Lakon (1942) who exposed seeds to selenium salts and then to tetrazolium salts. Lakon found that the tetrazolium salts method was more effective. This test helped distinguish viable and dead tissues in the embryo based on respiration rates. Seeds should be soaked in water prior to the tetrazolium treatment to allow hydration of the tissues. Tetrazolium solution can then be added directly to the seed, and stored in an oven for

2 hours at 95 °F for a complete coloration to determine viability. To determine results, it is necessary to view carefully the staining pattern (Copeland, 1976). Research conducted by Aslam et al., (2010) exposed seeds of Blue pine/Kail [*Pinus wallichiana* (A.B Jackson)] to the tetrazolium test. Seeds were soaked in distilled water for a period of 24 hours before being exposed to 1% solution of tetrazolium salt and stored in a dark room for 36 hours at a temperature of 86 °F \pm 5. After 36 hours seeds whose embryos stained a reddish color were classified as viable. The tetrazolium test helps differentiate living cells from dead cells, simply by identifying living cells as the ones that pick up the tetrazolium dye, and become stained a reddish color. Thus, viable seeds become stained, and non-viable seeds with non-living cells remain colorless (Aslam et al., 2010).

Seed Dormancy

Dormancy in seeds may inhibit germination. There are physical and physiological factors affecting dormancy. The most common dormancy mechanism is an impermeable seed coat. Breaking hard seed coats may occur naturally in time by wetting, drying, and freezing, or by passing through the digestive tract of an animal (Copeland, 1976).

In the nursery industry, to overcome seed coat impermeability, seeds are often scarified to allow faster germination and uniform imbibition (Copeland, 1976). There are different methods of scarification. Mechanical scarification can be accomplished by rubbing the seeds against an abrasive surface. Chemical scarification can be done with sulfuric acid, hydrochloric acid, acetone or alcohol, and hot water (Copeland, 1976). Sulfuric acid is used for thick seed coats that surround the embryo. After scarification, seeds need to be soaked in water, so that oxygen and water become available to the seed and germination can take place (Luna, et al., 2009).

For buffaloberry seeds, studies recommend acid scarification and cold stratification treatments (Rosner and Harrington, 2003). They also showed that scarification with sulfuric acid for 0-5 min and cold stratification for 98 days, resulted in increased germination from 27% - 38% (Rosner and Harrington, 2003).

In moist stratification for periods longer than one month, seeds could be exposed to bacteria and fungi leading to low germination percentages. Seeds should be surface sterilized in a solution of 300 ppm of trichlor (trichloro-s- traizinetrienes, Pool Time Products, Buford, GA) for 24 hours (Greer and Rinehart, 2010). Also, stratifying seeds has some advantages such as hastening germination and increasing seedling uniformity which is desirable for nursery production (Luna, et al., 2009).

Use of Gibberellic Acid

Gibberellic acid (GA_3) is a naturally occurring plant hormone that can release seeds from dormancy (Standturf, 2010). The positive effect of GA_3 is that it promotes uniform seed germination and increases germination percentages (Standturf, 2010). Gibberellic acid removes physiological dormancy mechanisms that often require lengthy stratification or light to maximize germination (Copeland, 1976). Gibberellic acid is an important plant hormone used for the regulation of internal seed dormancy in species with underdeveloped embryos (Luna, et al., 2009). Seeds of red elm, grey alder and buffaloberry exhibit unknown dormancy issues which is the reason for this investigation.

Softwood Cuttings

According to Pijut et al., (2011), softwood cuttings need to be collected and prepared from spring to summer. Softwood cuttings of many species root easily compared to other types

of cuttings. However, they are perishable and easily stressed. It is recommended to collect the cuttings in early morning and to keep them moist and cool before any further treatment. Leaves from selected cuttings should be small in size for low transpiration rates (Pijut, et al., 2011).

Hardwood Cuttings

Hardwood cuttings may lose moisture during collection, while being cut or stored. Cuttings should be mature enough to withstand inserting into the growing medium without breaking (Mainland, 1993). Hardwood cuttings should always be kept cool, out of direct sunlight, and can be stored for a few days near 32 °F. Research reports successful rooting from forest tree species such as alder (*Alnus* spp.), ash (*Fraxinus* spp.), basswood (*Tilia* spp.), and elm (*Ulmus* spp.) when optimum conditions are met. These conditions include: taking basal cuts below the node, applying a rooting hormone on the basal end of the cutting, increasing media temperature, decreasing atmospheric temperature, and exposing cuttings to light and mist when propagated in the greenhouse (Pijut, et al., 2011).

Many factors should be considered when propagating by cuttings. Some of these include plant characteristics such as, juvenility, cutting type, presence of leaves, buds, etc.; and environmental characteristics such as, humidity, light, heat, and photoperiod. Each of these factors or combinations of them may significantly affect the rooting response (Couvillon, 1988). Previous research has concluded that by increasing day length of Rabbiteye blueberry [*Vaccinium ashei* (J.M Reade)] cuttings exposed in the bed may increase the percent rooting and root quality (Couvillon, 1988).

Plant Hormones in Cutting Propagation

Plant hormones are naturally occurring organic compounds that, in low concentrations, control growth in plants. Plant growth regulators (PGRs) include both hormones and synthetic hormone-like compounds. Auxins make up one group of PGRs that have been used commercially for over 50 years to inhibit fruit set and leaf abscission (drop), promote flowering, reduce suckering, and initiate adventitious rooting of stem cuttings (Taiz and Zeiger, 2002). Auxins used as root-promoting compounds hasten root development, increase rooting uniformity, and encourage rooting of hard-to-root species. Common examples of auxins are indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA).

IBA in its acid form is insoluble in water, so it must be dissolved in an organic solvent (such as alcohol), or chemically modified to produce a water-soluble form. A pH buffer may also be included in commercial formulations. One specific type of water-soluble IBA that has been formulated is the potassium (K) salt of IBA and can be easily dissolved in water (Blythe, et.al., 2007).

During the past few years, growers in the U.S. have experienced difficulty in purchasing K-IBA from scientific supply companies for producing "rooting hormone" solutions. No commercial rooting products containing K-IBA have been registered with the EPA, due in large part to the high cost of registration. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), only registered products may be used by growers.

IBA dissolved in alcohol may be less effective than if dissolved in water, because high concentrations of alcohol may injure, dehydrate, or be toxic for plant tissue, stems and scions (Kroin, 1992). Further, alcohol is a solution that evaporates quickly. When alcohol evaporates, the ppm of IBA concentration eventually increases, which may become phytotoxic (Kroin, 1992)

Alternatives to K-IBA include: water-soluble salts of IBA, dilutable concentrates which are usually formed in 1:10 ratio IBA+NAA to water for example Dip'N Grow, or powder (talc) formulations of IBA. Water-soluble salts can typically be substituted for K-IBA at the same concentration as was previously used with K-IBA. However, when substituting a solution prepared from a concentrate, a lower concentration of IBA may be appropriate because commercial concentrates also contain NAA. If using an IBA powder in place of a K-IBA solution, a similar or higher concentration of IBA may be needed. IBA may not be taken up as readily from the powder, even though the powder remains in contact with the base of a cutting for a longer time compared with a liquid solution. Therefore, some trialing may be needed if switching from K-IBA to the use of an IBA+NAA concentrate or an IBA powder.

Bottom Heat

The use of bottom heat in hardwood cuttings can be a helpful tool. Considerations while using bottom heat may include: the need for increased watering due to the higher soil temperature that will be constantly maintained, covering flats with plastic to slow the evaporation during the rooting process, and providing young plants with light to keep new growth compact (Phytotronics, Inc., 2011).

Objective of Research

The objective of this project was to find techniques which improve the commercial propagation of red elm, grey alder, and buffaloberry for the purpose of enabling tribes and land owners to increase the presence of these native plants on their lands. Determine if GA₃ (gibberellic acid) can improve germination rates in red elm, grey alder, and buffaloberry, and

evaluate the use of K-IBA and Dip 'N GrowTM for red elm, grey alder and buffaloberry cuttings as an alternative for propagation of these species.

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USDA NRCS, 2011. *Shepherdia canadensis* (L.) Nutt. USDA . Accessed October 8, 2011.
http://plants.usda.gov/plantguide/pdf/cs_shca.pdf

Figures and Tables

Figure 1.1 Red Elm (*Ulmus rubra*) characteristics a) trunk cross section , b) reddish brown color noticeable on the bark, c) seeds d) leaf.



a)



b)

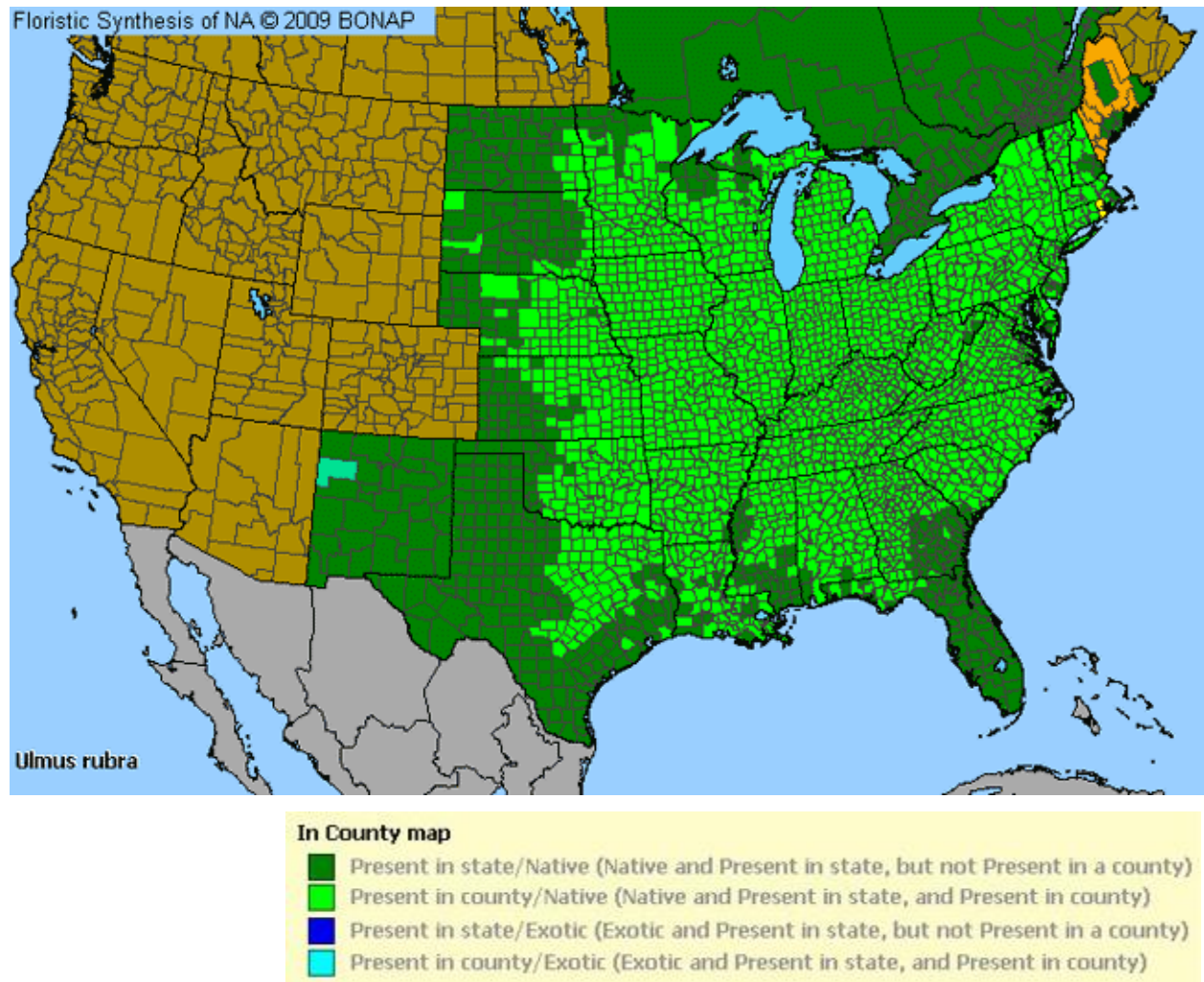


c)



d)

Figure 1.2 Distribution map of red elm (*Ulmus rubra*) in the United States.



(IMS Health Incorporated, 2012)

Figure 1.3 Commercial uses of red elm (*Ulmus rubra*), includes a current product Throat Comfort Tea.

YOGI TEA® HEALING FORMULA		
Honey Lemon Throat Comfort® Throat Health Support CERTIFIED ORGANIC		
Supplement Facts Serving Size 1 tea bag (Makes 8 fl oz)		
	Amount Per Tea Bag	Daily Value
Proprietary Blend of Herbs	2000 mg	
Organic Honeybush Leaf		†
Organic Lemon Grass Leaf		†
Organic Lemon Myrtle Leaf		†
Organic Licorice Root		†
Organic Peppermint Leaf		†
Organic Black Cherry Bark		†
Organic Echinacea Root (Angustifolia, Purpurea, Pallida)		†
Organic Black Pepper		†
Organic Slippery Elm Bark		†
Stevia Leaf		†
† Daily Value not established.		
Other Ingredients: Organic Lemon Flavor, and Natural Honey Flavor. Caffeine Free		
YOGI TEA® Certified Organic by QAI, Inc.		
HERBAL DIETARY SUPPLEMENT		



(Yogi tea, QAI, Inc.)

Figure 1.4 Grey Alder (*Alnus incana*) characteristics a) cross section b) bark c) leaf d) seeds.



a)



b)



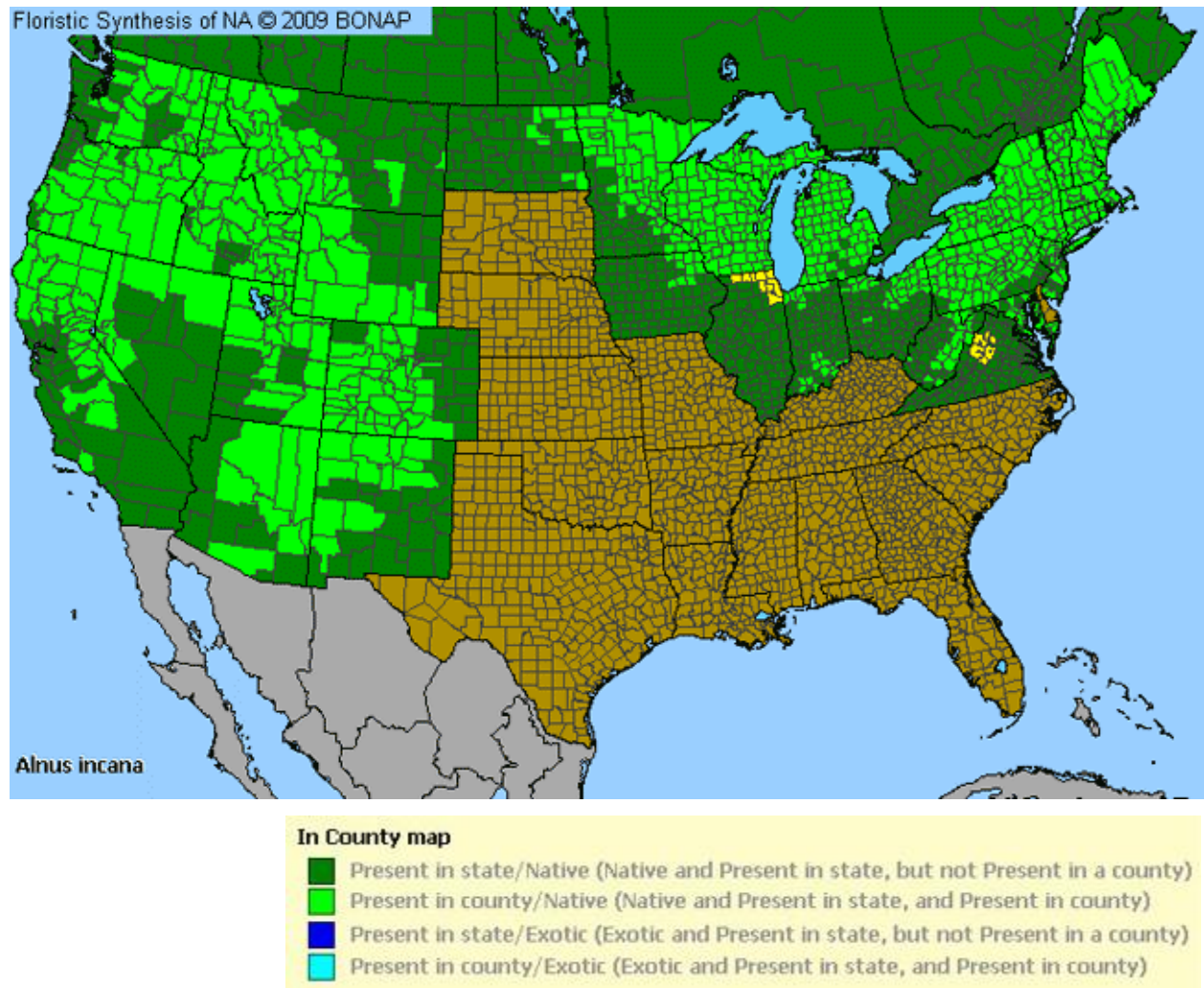
c)



d)

(United States Department of Agriculture NRCS, 2012).

Figure 1.5 Distribution map of Grey Alder (*Alnus incana*) in the United States.



(IMS Health Incorporated, 2012).

Figure 1.6 Buffaloberry (*Shepherdia canadensis*) characteristics a) shrub b) fruit c) leaf d) seeds.



a)



b)



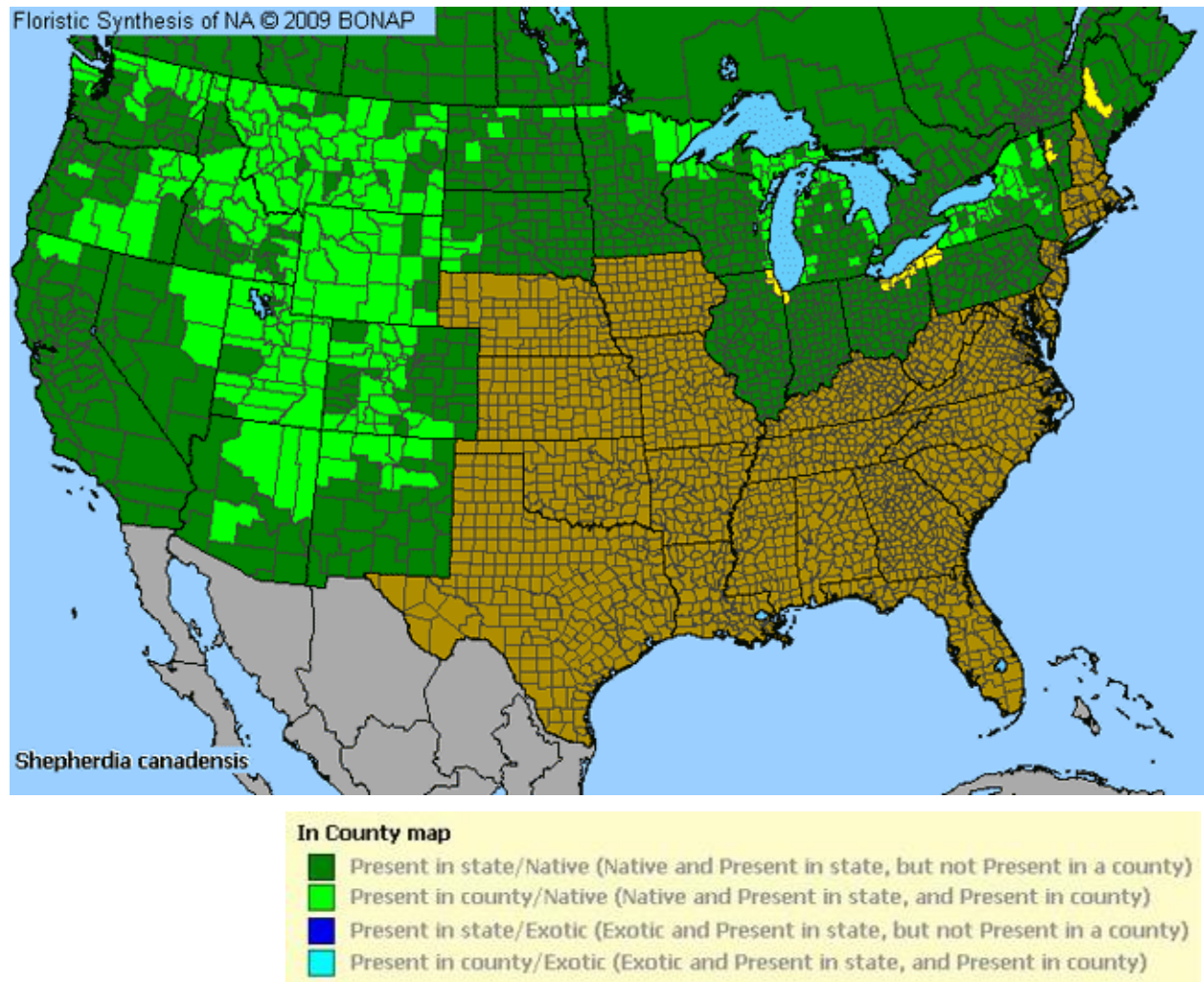
c)



d)

(USDA, 2012)

Figure 1.7 Distribution map of Buffaloberry (*Shepherdia canadensis*) in the United States.



(IMS Health Incorporated, 2012).

Chapter 2 - Seed Propagation of Red Elm (*Ulmus rubra*), Grey Alder (*Alnus incana*), Mountain Alder [*A. incana* spp. *tenuifolia*] and Buffaloberry (*Shepherdia canadensis*) using Gibberellic Acid Treatments

Abstract

Red elm (*Ulmus rubra*), grey alder (*Alnus incana*), mountain alder [*Alnus incana* and *A. incana* spp. *tenuifolia*] and buffaloberry (*Shepherdia canadensis*) are considered important plants for many Native American tribes in the United States. Native Americans have used these three species for a variety of medicinal uses and ceremonial purposes. Red elm is valued as a ceremonial tree, but it is susceptible to Dutch Elm Disease caused by the fungus (*Ophiostoma ulmi*) and is not grown in many ornamental nurseries. Grey alder and buffaloberry are nitrogen fixing trees that help improve soil fertility. Currently, Kansas tribal leaders would like to plant more of these species on tribal land, but the plants have been difficult to propagate. The objective of this study was to evaluate the effect of using gibberellic acid (GA₃), a naturally occurring plant hormone in different concentrations until reaching the optimum level needed for maximum germination of red elm, grey alder and buffaloberry seeds. This will lead to an increase in the production of the species commercially and enable tribes and land owners to increase the presence of these native plants on their lands. Studies were conducted with stratified (cold, moist storage) and non-stratified red elm, grey alder, and buffaloberry seeds soaked in one of four treatments: 0, 250, 500 or 1000 ppm of GA₃ in 2010, and 0, 500, 1000, 2000 ppm of GA₃ in 2011. Results indicate the use of GA₃ in high concentrations promoted germination of unstratified seeds of red elm, though low seed viability in grey alder and buffaloberry resulted in poor germination, regardless of treatments.

Introduction

Red Elm (*Ulmus rubra*), also called slippery elm, is a native North American tree that is valued by many American Indian tribes as fuel for ceremonial fires at pow wows, funerals and sweat lodges. Other past uses of red elm included using the inner bark for cordage, fiber bags and storage baskets. In spring the cambium becomes very mucilaginous, which was used for several medicinal purposes, including as a treatment for swollen glands, as eyewash, for sore throats and women drank a tea of the bark to make childbirth easier (Anderson, 2010). Current tribal use centers on using red elm for firewood in traditional ceremonies.

Grey alder (*Alnus incana*), also called tag alder, mountain alder, or hazel alder, is a species of moist lowlands, common in the region surrounding the Great Lakes including east-central Canada, south to Virginia and Maryland. Native Americans used alder to treat anemia, for internal bleeding, urinary problems, bruises, backaches, and skin irritations (Anderson, 2010). It is used locally for fuel, and also supports symbiotic nitrogen-fixing bacteria in root nodules, which makes the alder valuable for improving soil fertility. Alders are also used as landscape ornamentals (USDA, 2011).

Sub-species of grey alder, *Alnus incana* ssp. *tenuifolia* (Nutt.), also known as thin leaf alder, has similar characteristics to *Alnus incana* except that it is considered a shrub or small tree, 20 -30 ft in height that grows throughout the mountain ranges of western North America. Seeds exhibit dormancy which can be broken by cold stratification (USDA, 2011).

Buffaloberry (*Shepherdia canadensis*) is also called soapberry, russet red buffaloberry or Canadian buffaloberry. It is a native, deciduous, nitrogen-fixing shrub from Alaska to Maine and south from New York to South Dakota and south at higher elevations to Arizona. Buffaloberry fruits are eaten fresh or dried and also used to make “Indian ice cream”. Berry juice was used to

prevent heart attacks and indigestion. The berries were also chewed to induce childbirth (USDA, 2011).

Gibberellic acid (GA₃) is a naturally occurring plant hormone that can release seeds from dormancy. The positive effect of GA₃ is that it promotes uniform seed germination and increases germination percentages (Adams et al., 2010). Gibberellic acid removes physiological dormancy mechanisms that often require lengthy stratification or light to maximize germination (Norden et al., 2007). Seeds of red elm, grey alder and buffaloberry exhibit unknown dormancy issues and thus are the subject of this investigation.

The objective of this study was to evaluate the effect of using GA₃ (a naturally occurring plant hormone that can release seeds from dormancy and promote uniform seedling germination) in different concentrations until reaching the optimum level needed for maximum germination of red elm, grey alder and buffaloberry seeds. These will lead to an increase in the production of the species commercially and enable tribes and land owners to increase the presence of these native plants on their lands.

Materials and Methods

2010 Study

To determine seed viability, a tetrazolium test was done by using 25 seeds from each species: red elm, grey alder and buffaloberry (Fig. 2.1). Seeds were soaked in 100 ml of distilled water at room temperature exposed to daylight for a period of 24 hours to allow hydration of the tissues. Seeds were then cut in a longitudinal way to expose the embryo to allow observation of the stain, and triphenyl tetrazolium chloride mix was added (Sigma-Aldrich Co. LLC, US) with distilled water made at 1% solution (1g/100 ml). Seeds were then incubated at 30 °C± 5 in

darkness for a period of 36 hours. Viability results were accomplished when seeds either stained pink (respiring tissues) or didn't change color. Data collected included the number of seeds with respiring tissues and thus percentage of viable seeds were calculated.

Seeds from red elm, grey alder, and buffaloberry were used in the study. Red elm seeds were collected from two locations: Butler County (Jackman State Forest), and Douglas County (Prairie Park), Kansas in April 2010 (Fig. 2.2). Seeds from grey alder and buffaloberry plants were obtained from Lawyers Nursery (Montana) .

Buffaloberry seeds were scarified using sulfuric acid (SO_4) for 20 minutes and rinsed with tap water and then air dried for 1 hour, prior to stratification. All seeds were divided into two treatments (stratified and unstratified), air-dried for three days and stored in sealed glass containers. Immediately after scarification, seeds received cool moist stratification at 5 °C for 90 days. Stratification was accomplished by placing 60 seeds per pouch (fabric bag) in one polyethylene bag containing 454 g of moist peatmoss. The remaining 240 seeds were immediately treated with GA_3 (Research Organics, Cleveland, OH) at concentrations of 0, 250, 500, 1000 ppm (Fig. 2.3). Sixty seeds were placed in beakers containing 120 ml of each concentration GA_3 solution and placed on a shaker (Innova 2100 , New Brunswick Scientific CO., Inc, Edison, NJ) at 175 rpm (revolutions per minute) for 24 hours (Fig. 2.4). Seeds were then placed on moist filter paper inside 47-mm-diameter petri dishes (Fisher Scientific, USA) using 2 ml of distilled water to maintain humidity. Petri dishes were placed on a lab bench at room temperature ($18^\circ\text{C}\pm 5$) until radicle emergence. Light was on the lab for a period of 8 hours a day. Stratified seeds were handled identically upon removal from the fabric pouches after 90 days.

Germination was monitored every three days and recorded when emerged radicles reached a length of 3 mm. Data was collected over a period of 2 weeks, after germination began and seeds in all treatments had either germinated or appeared dead. The experimental design was a randomized complete block with a 2×4 factorial arrangement of treatments (Fig. 2.5). There were two stratification treatments (no stratification or stratification) and four GA₃ treatments (0, 250, 500, or 1000 ppm). The experiment was replicated six times using two petri dishes per replicate for each treatment with five seeds per petri dish. Data was subjected to ANOVA (Statistical Analysis System, SAS Institute Inc., Cary, NC) and means separation by LSD test ($p < 0.05$).

2011 Study

The same procedure was conducted for the seeds collected and treated in 2011. The only variations were in GA₃ treatment concentrations, which were increased, and stratification period was reduced from 90 days to 45 days. Unstratified and stratified seeds of red elm were soaked for 24 hours in solutions of 0, 500, 1000, or 2000 ppm of GA₃. For grey alder and buffaloberry, the study did not continue due to poor viability among seeds.

Results and Discussion

2010 Results

Viability was much higher for red elm than for grey alder or buffaloberry, nevertheless viability results were similar from year to year. Seeds of red elm collected in 2010 were 94% viable, while seeds collected in 2011 resulted in 80% viability. However, grey alder (*A. incana*), and buffaloberry seeds resulted in poor viability. Seeds from both species of grey alder only had

4% viability for year 2010 and 2011. Buffaloberry seeds were only 4% viable in 2010 and 0% in 2011, which is consistent with the literature from the USDA (2011).

Unstratified red elm seeds collected from Butler County exhibited greater germination rates among all treatments (Fig 2.6, Fig 2.7). Both seed lots exhibited similar trends in germination across the various treatments. Douglas County seed lot samples showed the best treatment for the unstratified seeds at 1000 ppm GA₃, which had a 78% germination rate. Germination percent steadily increased from the control GA₃ treatment (0 ppm) with 13%, to 28% for 250 ppm GA₃, and 43% for 500 ppm GA₃. Conversely, stratified seeds resulted in optimal germination at 0 ppm of GA₃ with 31% and declining germination with increasing concentrations of GA₃. Stratified seeds from the Douglas County seed lot exposed to 1000 ppm GA₃ resulted in just 6% germination rate, significantly lower than all other treatments.

Butler County seed lot samples, showed similar results at both: 1000 ppm GA₃ (88%) and 500 ppm GA₃ (77%) for germination with unstratified seeds (Figure 2.6). Germination decreased with decreasing GA₃ concentrations with 250 ppm GA₃ having 52%, and 0 ppm GA₃ having 30% germination. Conversely, stratified seeds had higher germination rates at 0 ppm GA₃ with 47% germination rate and significantly lower germination percent for seeds treated with higher GA₃ rates. Similar to the Douglas County seed lot, stratified seed exposed to 1000 ppm GA₃ had 3% germination rates, which was the lowest of all treatments.

Unlike red elm, grey alder (*Alnus incana*) experienced poor germination with and without stratification, among all GA₃ treatments (Table. 2.1). However, the trend of lower germination percentages when combining stratification with GA₃ treatment was similar for both species. Unstratified seeds of grey alder treated at 1000 ppm GA₃ had 10%, follow by 8% germination for 250 ppm, while unstratified seeds treated with 0 ppm GA₃ (control) and 500 ppm GA₃ each had

3% germination. Stratified seeds of grey alder experienced optimal germination at 0 ppm GA₃ with an average of 8%. Germination percentage declined with increasing GA₃ concentrations: 500 ppm GA₃ exhibited 5 % germination, 250 ppm GA₃ had 2 % germination, and 1000 ppm GA₃ had 3% germination (Table. 2.1). Results suggest that seed propagation of grey alder may not be a viable option for mass production of this species.

For *Alnus incana* ssp. *tenuifolia*, no trend was observed, nevertheless, there was poor germination in unstratified seeds at treatments of 250 ppm and 1000 ppm GA₃ resulting in only 8% germination rate. The control treatment had 5% germination rate, followed by 3% germination rate respectively for the 500 ppm GA₃ treatment. Stratified seeds germination rates were observed at 8% in the control treatment (0 ppm GA₃), followed by 7% in the 250 ppm GA₃ treatment, 3% germination rate at 1000 ppm, and 2% at 500 ppm (Table. 2.2).

Unstratified seeds of buffaloberry had similar results to the grey alder seeds, in that at 0 ppm and 250 ppm GA₃ resulted in the same germination rate (5%). Unstratified seeds treated with 500 ppm and 1000 ppm (control) GA₃ had 2% germination rate. Conversely, for stratified seeds, the optimal germination rate was at 0 ppm GA₃ (control) with 7% germination and at 500 ppm GA₃ with 3%. At 250 ppm and 1000 ppm GA₃, no germination was observed (Table. 2.3). Rosner (2003) found in his study, that optimization of acid scarification and stratification combinations for russet buffaloberry seeds was obtained with a combination of 5 minute soak in sulfuric acid and 98 days of stratification, which improved germination by 11%.

Germination of unstratified red elm seeds with GA₃ was maximized at 1000 ppm. Previous work by Dirr and Heuser (2006) indicated that stratification would increase seed germination. While this was true for the control treatment not exposed to GA₃, treatments with

GA₃ resulted in significantly less germination for 90-day stratified seeds when compared with unstratified seeds.

Unstratified seeds obtained from Douglas County showed no significant differences within or between treatments. Overall, germination was similar for all treatments. At 500 and 2000 ppm of GA₃, 30% of the seeds germinated, while 25% resulted from the 1000 ppm GA₃ treatment. Though no trend was observed, 0 ppm GA₃ (control treatment) had the best germination rate at 35% (Table. 2.5). Stratified seeds results were: 0 ppm (control treatment) had 38% germination, 500 and 2000 ppm of GA₃ had 47% while the 1000 ppm was the highest germination rate with 50%.

Unstratified seeds from the Butler County seed lot resulted in 28% germination rate at 0 ppm, 40% at 500 ppm, 35% at 1000 ppm and 57% at 2000 ppm of GA₃ treatment. Stratified seeds had similar results with the best treatment which was 2000 ppm GA₃ which had a 90% germination rate. Germination was similar for other rates from the 1000 ppm of GA₃ treatment at 88% germination, to 72 % germination for 500 ppm GA₃, and 65% germination for 0 ppm (control treatment) GA₃ (Table 2.5). Increasing concentrations of GA₃ for the non- stratified seeds resulted in increased germination rates.

In general for all species, gibberellic acid treatment had a positive effect on germination percentage of unstratified seeds. This suggests that there may be an optimal response of stratification for red elm seeds treated with GA₃. Unstratified seeds benefited from exposure to exogenous GA₃, while stratified seeds (which naturally produce endogenous GA₃) experienced a variable and perhaps inhibitory response when exposed to additional GA₃. This relationship needs further investigation.

There was no significant difference for grey alder seeds among all the GA₃ treatments. Previous work by Earl (2002) suggested that grey alder seeds require stratification in moist sand for 60 to 90 days.

Buffaloberry seeds showed no significant differences among treatments and had very low germination rates. Dirr and Heuser (1987) recommended scarification for 20 to 30 minutes followed by a period of two to three months of stratification. Another study (Krishnan and Harrison, 1991) recommends that buffaloberry should be propagated by cuttings due to the low viability of the seeds.

A possible side effect from applying excess GA₃ to induce germination is it may produce changes in the shape of the leaf (Evans, 1996). Nevertheless it has been shown that seeds of mountain camellia (*Stewardia ovata*) soaked in a GA₃ treatment for 24 hours and seven months of cold stratification (39 °F) will exhibit increased germination (Nair and Zhang, 2010). No unusual leaf morphology was noted in seedlings grown from the previously mentioned 2010 study.

Conclusions

Based on this study, we recommend unstratified red elm seeds to be soaked in 1000 ppm GA₃ for 24 hours before sowing. Alternatively a short stratification of 45 days could be used again with 1000 ppm of GA₃. Seeds typically took 10-15 days to germinate. The second year study (2011) which had higher concentration of GA₃ treatments (0, 500, 1000, 2000 ppm), showed maximum germination rates for the red elm stratified seeds at 2000 ppm GA₃, yet above 1000 ppm, the trend started to decline. For the grey alder (*Alnus incana*, *A. incana* spp *tenuifolia*),

even though we increased the amount of GA₃ for both stratified and unstratified seeds, we did not obtain satisfactory results.

Recommendations

Future studies are needed to evaluate environmental treatments, such as: exposure to light, days of stratification, and temperature, which can affect seed germination. Additional studies will examine rooting capacity with dormant and greenwood cuttings of the three species evaluated in this study.

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http://plants.usda.gov/plantguide/pdf/cs_alinr.pdf
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http://plants.usda.gov/plantguide/pdf/cs_shca.pdf

Figures and Tables

Figure 2.1 Red elm, grey alder, and buffaloberry seeds used in the seed germination study.



Figure 2.2 Locations is Kansas (Butler and Douglas counties) where seed of red elm was collected.

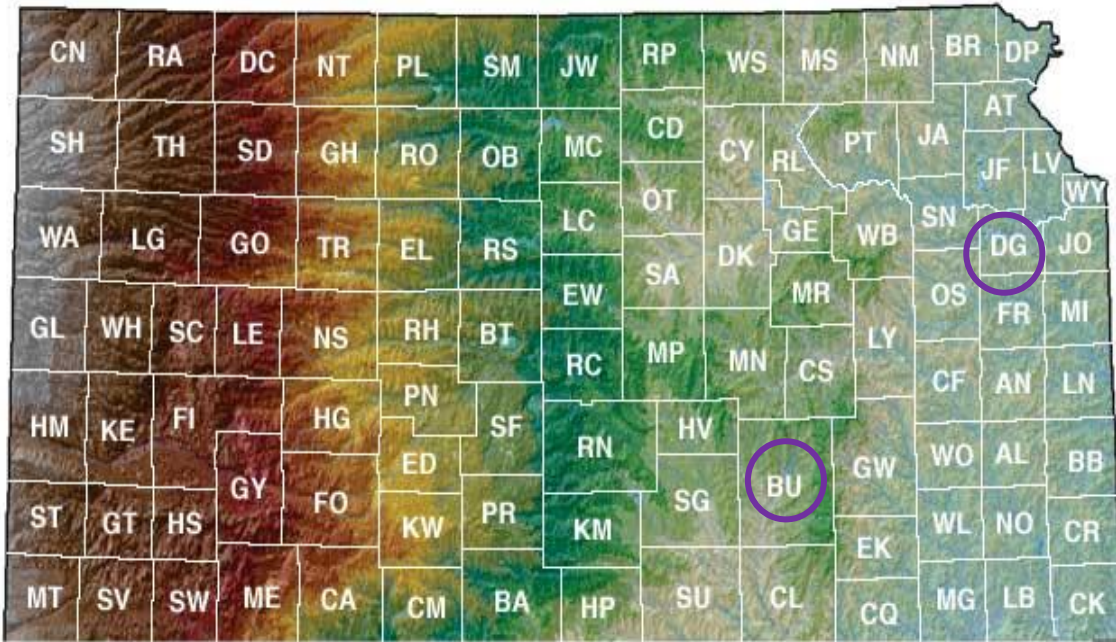


Figure 2.3 Seeds were treated with gibberellic acid (GA_3) in 150 ml beakers, and placed on a shaker table at 175 revolutions per minute (rpm) for 24 hours.

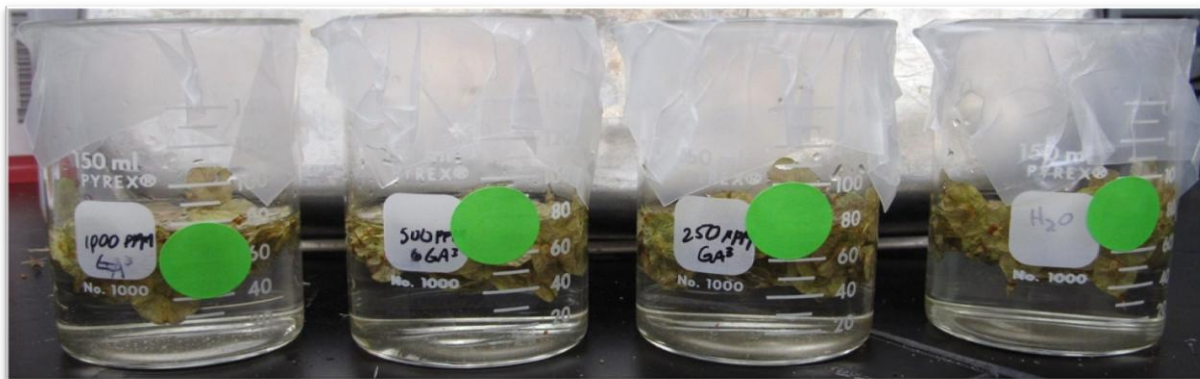


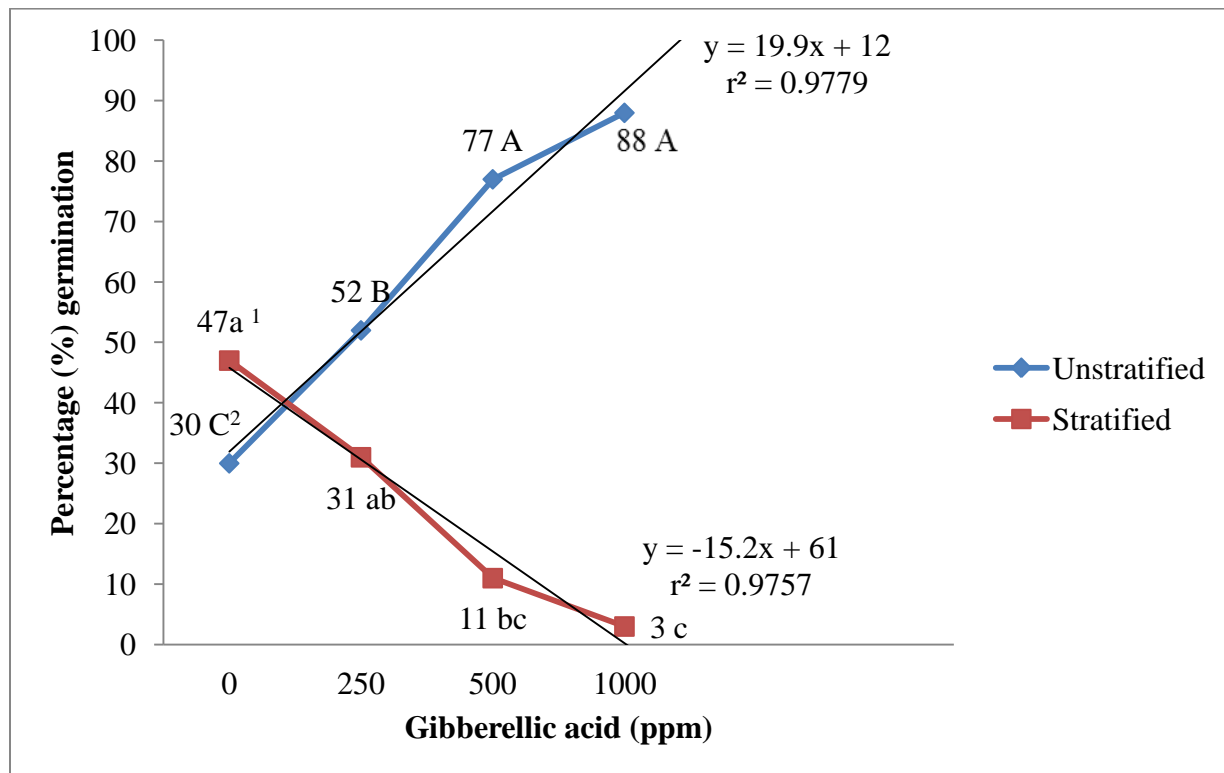
Figure 2.4 Seeds were placed in beakers containing 120 ml of GA₃ solution and placed on a shaker (Innova 2100 , New Brunswick Scientific CO., Inc, Edison, NJ) at 175 rpm (revolutions per minute) for 24 hours.



Figure 2.5 The experimental design was a randomized complete block with a 2×4 arrangement of factorial treatments.



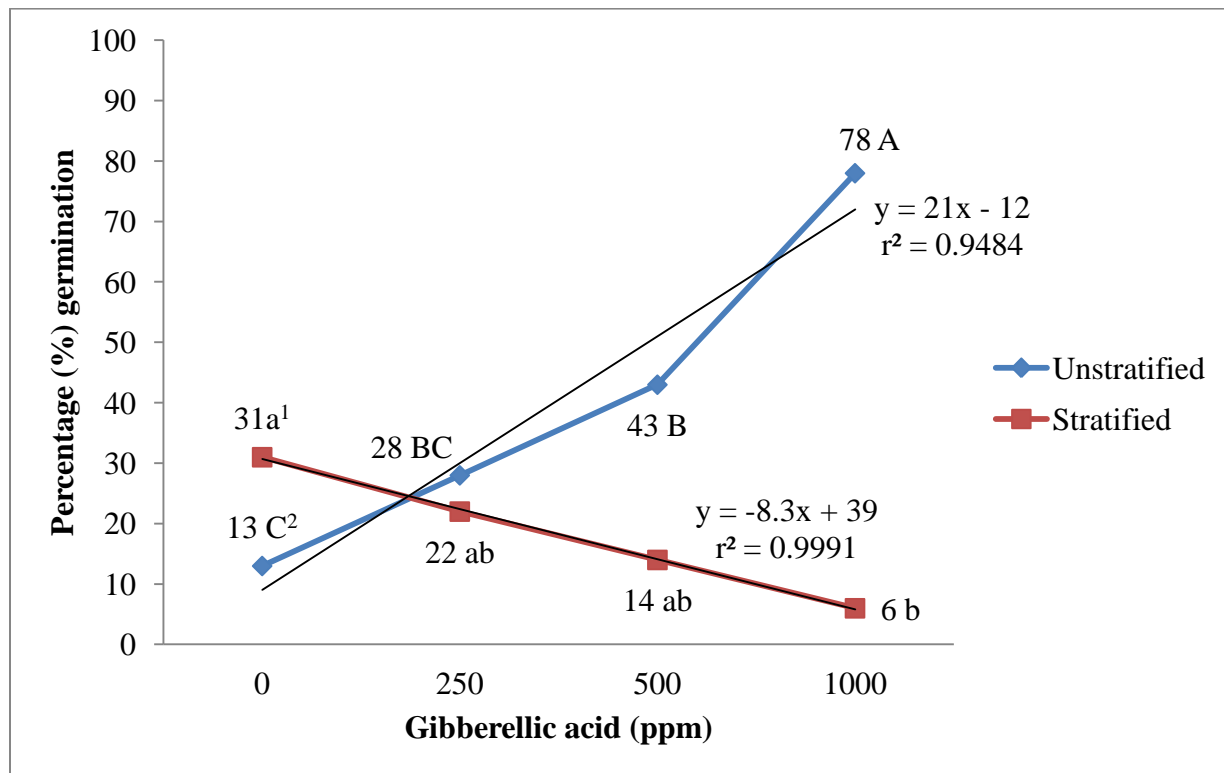
Figure 2.6 Germination percentages of red elm (*Ulmus rubra*) treated with GA₃ in 2010, seed collected at Butler County.



¹ lower case letters represent significant difference within stratified treatments.

² upper case letters represent significant difference within unstratified treatments.

Figure 2.7 Germination percentages of red elm (*Ulmus rubra*) treated with GA₃ in 2010, seed collected at Douglas County.



¹ lower case letters represent significant difference within stratified treatments.

² upper case letters represent significant difference within unstratified treatments.

Table 2.1 Percentage germination of grey alder (*Alnus incana*) seeds treated with GA₃ in 2010.

GA ₃ concentration (ppm)	Treatments	
	Unstratified (%)	Stratified (%)
0	3 A	8 A
250	8 A	5 A
500	3 A	2 A
1000	10 A	3 B

No significant difference at LSD (p<0.05; n=6)

¹ upper case letters represent significant difference between sites, overall means by site and stratified and unstratified treatment.

Table 2.2 Percentage germination of grey alder (*Alnus tenuifolia*) seeds treated with GA₃ in 2010.

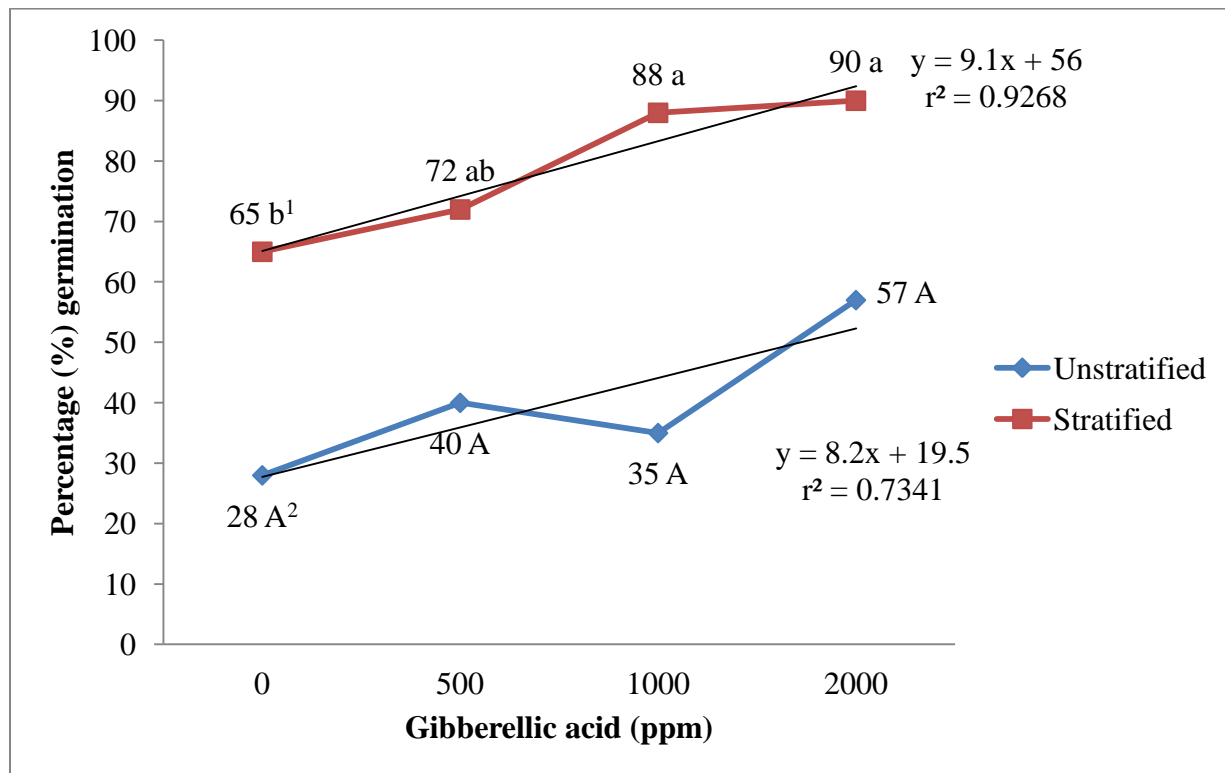
GA ₃ concentration (ppm)	Treatments	
	Unstratified (%)	Stratified (%)
0	5	8
250	8	7
500	3	2
1000	8	3

No significant difference at LSD (p<0.05; n=6)

Table 2.3 Percentage germination of buffaloberry (*Shepherdia canadensis*) seeds treated with GA₃ in 2010.

GA ₃ concentration (ppm)	Treatments	
	Unstratified (%)	Stratified (%)
0	5	7
250	5	0
500	2	3
1000	2	0
No significant difference at LSD (p<0.05; n=6)		

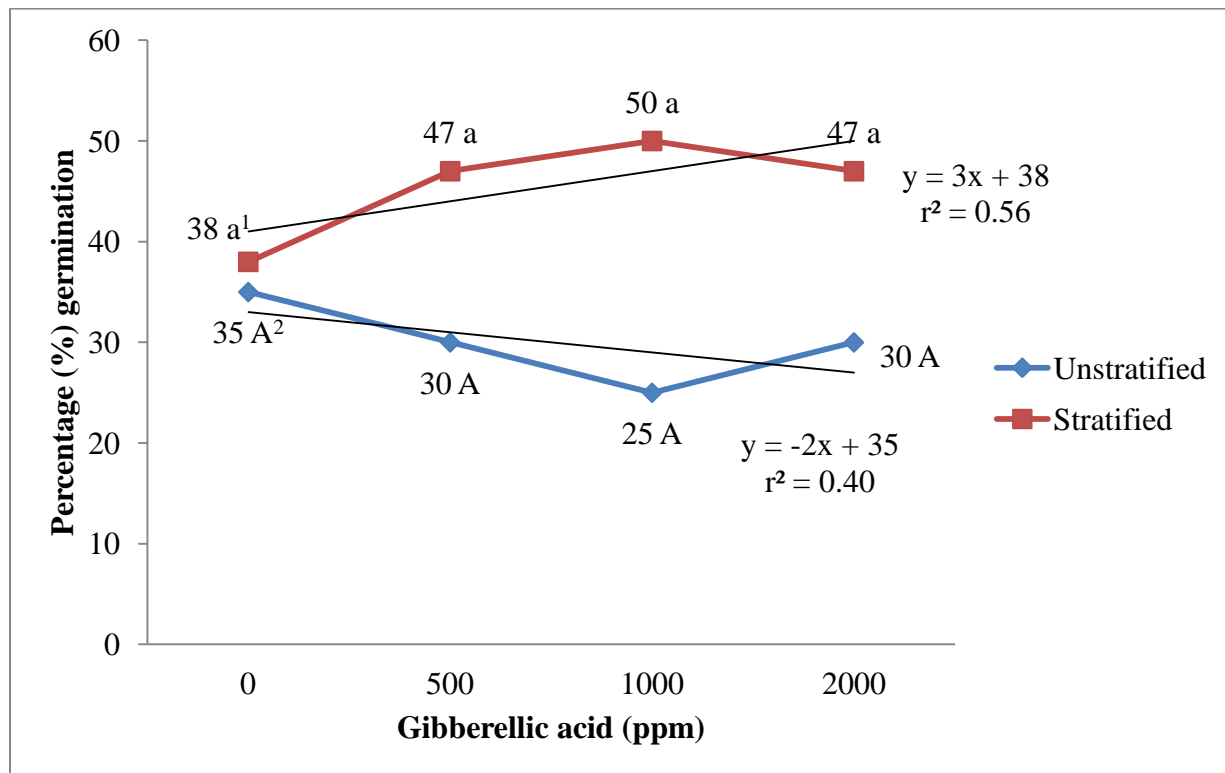
Figure 2.8 Germination percentages of red elm (*Ulmus rubra*) treated with GA₃ in 2011, seed collected at Butler County.



¹ lower case letters represent significant difference within stratified treatments.

² upper case letters represent significant difference within unstratified treatments.

Figure 2.9 Germination percentages of red elm (*Ulmus rubra*) treated with GA₃ in 2011, seed collected at Douglas County.



¹ lower case letters represent significant difference within stratified treatments.

² upper case letters represent significant difference within unstratified treatments.

Chapter 3 - Propagation of Red Elm (*Ulmus rubra*), Grey Alder (*Alnus incana*) and Buffaloberry (*Shepherdia canadensis*) by hardwood and softwood cuttings using K-IBA and Dip' N Grow

Abstract

Native American tribes are interested in planting species which are naturally declining that have medicinal value. Three different species, red elm (*Ulmus rubra*), grey alder (*Alnus incana*) and buffaloberry (*Shepherdia canadensis*) were selected for study. The objective of this research is to find alternatives to propagate these species. We evaluated the use of K-IBA (Potassium indole-3-butyric acid) and Dip 'N GrowTM (Indole-3-butyric acid + naphthalene acetic acid) for initiating roots on red elm, grey alder and buffaloberry stem cuttings. Softwood cuttings for the three species were treated with K-IBA at 5,000 and 10,000 ppm and Dip 'N GrowTM at 1:10 ratio solutions. Data was collected after 12 weeks. Results of this study showed that only grey alder softwood cuttings had callus formation, root growth, and shoot growth while red elm and buffaloberry did not respond to cutting propagation treatments. We did observe that red elm tip cuttings remained alive, but no shoot or root formation appeared.

Introduction

Red Elm (*Ulmus rubra*), also known as slippery elm, is a native North American tree that is valued by many American Indian tribes as fuel for ceremonial fires at pow wows, funerals and sweat lodges. In the past, the inner bark of red elm was used to make cordage, fiber bags and storage baskets. It was used for several medicinal purposes, including as a treatment for swollen glands, as eyewash, for sore throats and women drank a tea of the bark to make childbirth easier (Anderson, 2010). Current tribal use centers on using red elm for firewood in traditional

ceremonies which is characterized to be the preferred wood for their fire ceremonies because it is much denser than the american elm. Currently there is a tea (throat comfort) sold in the market which is recommended for colds and cough.

Grey alder (*Alnus incana*), also known as called tag alder, mountain alder, or hazel alder, is a species of moist lowlands, common in the region surrounding the Great Lakes including east-central Canada, south to Virginia and Maryland. Native Americans used alder to treat anemia, for internal bleeding, urinary problems, bruises, backaches, and skin irritations (Anderson, 2010). It is used locally for fuel, and also supports symbiotic nitrogen-fixing bacteria in root nodules, which makes the alder valuable for improving soil fertility. Alders are also used as landscape ornamentals (USDA, 2011).

Buffaloberry (*Shepherdia canadensis*) is also known as soapberry, russet red buffaloberry or canadian buffaloberry. It is a native, deciduous, nitrogen-fixing shrub found from Alaska to Maine and south from New York to South Dakota and at higher elevations to Arizona. Buffaloberry fruits are eaten fresh or dried and also used to make “Indian ice cream”. Berry juice was used to prevent heart attacks and indigestion. The berries were also chewed to induce childbirth (USDA, 2011). Currently you can find buffaloberry jelly which is sold commercially. The objective of the study is to evaluate the use of K-IBA and Dip ‘N Grow™ for red elm, grey alder and buffaloberry cuttings as an alternative for propagation of these species.

Commercial producers use vegetative propagation as means to clonally produce improved plant varieties. Aspects to take into consideration while propagating by cuttings are: juvenility, cutting type, presence of leaves, buds, and environmental characteristics such as: humidity, light, heat, and photoperiod. Each of these factors or combinations of them may significantly affect the rooting response (Couvillon, 1988).

According to Pijut et al. (2011), softwood cuttings need to be collected and prepared from spring to summer. Softwood cuttings root easily for some species compared to other types of cuttings. However, they are easily perishable and stressed. It is recommended to collect the cuttings in early morning and to keep them moist and cool before any further treatment. Leaves from selected cuttings should be small in size for low transpiration (Pijut et al., 2011).

Hardwood cuttings can also lose moisture during collection, while being cut or stored. Cuttings should be mature enough to withstand inserting into the growing media without breaking (Mainland, 1993). Hardwood cuttings should always be kept cool, out of direct sunlight, and can be stored for a few days near 32° F. Research reports successful rooting from forest tree species such as alder (*Alnus* spp.), ash (*Fraxinus* spp.), basswood (*Tilia* spp.), and elm (*Ulmus* spp.) when conditions are met. These conditions include: taking basal cuts below a node, applying a rooting hormone on the basal end of the cutting, increasing media temperature, decreasing atmospheric temperature, and exposing cuttings to light and mist when propagated in the greenhouse (Pijut et al., 2011).

Rooting of softwood and hardwood cuttings can be enhanced with a plant hormone for root initiation and for this we applied two plant hormone treatments, K-IBA (potassium salt of indole-3-butyric acid) and Dip 'N Grow™ (indole-3-butyric acid + naphthalene acetic acid) hormones at different rates.

The objective of this research was to evaluate the use of K-IBA (potassium salt of indole-3-butyric acid) and Dip 'N Grow™ (Indole-3-butyric acid+ naphthalene acetic acid) as alternative rooting hormones for red elm (*Ulmus rubra*), grey alder (*Alnus incana*) and buffaloberry (*Shepherdia canadensis*).

Materials and Methods

Experiment 1 Softwood Cuttings:

Cuttings were collected from the succulent new growth of red elm (*Ulmus rubra*) and grey alder (*Alnus incana*) from the tip, middle and terminal part of the branch. Cuttings were re-cut to approximately 6 in in length. The cuttings were then dipped into 10% hydrogen peroxide solution (to sterilize), and removed immediately. Proximal ends of the cuttings were re-cut on a 45° slant in with a sterilized knife. Hormone solutions were prepared in sterilized beakers with distilled water to create three concentrations (0, 5000, 10000 ppm) of K-IBA and a 1:10 ratio solution of Dip' N Grow™. Beakers were filled 1.6 in from the bottom with hormone and the cuttings were dipped for 1 second. Treated cuttings were then immediately stuck into the moist potting media. Cuttings were set under a mist bench in the greenhouse with the mist running 10 seconds every 20 minutes between 6:00 am to 6:00 pm for 12 weeks.

The experimental design was a randomized complete block with 4 hormone treatments (K-IBA and Dip' N Grow) and 6 replicates. Each replicate consisted of 4 stem cuttings (subsamples) per treatment. Data was collected for a period of twelve weeks and consisted of shoot length, number of cuttings with roots, dry weight callus, and total root length. Data was subjected to ANOVA (Statistical Analysis System, SAS Institute Inc., Cary, NC) and means separation by LSD test ($p < 0.05$).

Experiment 2 Hardwood Cuttings:

Procedure was done the same way as experiment 1. Treated cuttings were air dried for 5 minutes before being stuck into the moist potting media which was sealed inside a 1 lb polyethylene bag. The bag was set on a heat pad (Redi Heat Propagation Mats, Phytotronics Inc,

Earth city, MO) inside a cooler at $25^{\circ}\text{C} \pm 5$ with the cooler at 5°C . Cuttings were covered with black polyethylene bags to maintain humidity. Cuttings were watered once a week or when needed. The experimental design was randomized in complete blocks with 4 hormone treatments (K-IBA and Dip' N Grow) and 6 replicates. Each replicate consisted of 4 stem cuttings (subsamples) per treatment. Data was collected for a period of twelve weeks and consisted of shoot length, number of cuttings with roots, dry weight, callus, and total root length. Data was subjected to ANOVA (Statistical Analysis System, SAS Institute Inc., Cary, NC) and means separation by LSD test ($p < 0.05$).

Results and Discussion

Softwood cuttings:

After a period of 12 weeks, no significant results were observed for red elm between treatments or within treatments. We observed no callusing taking place, no root growth, or any sign that the cuttings were growing. Terminal cuttings were the only ones alive, yet like the rest of the cuttings, no callus formation or roots appeared at the wound.

For grey alder cuttings there was no significant difference in root length. Root length for treatment 1 (control) was 14.74 in, for treatment 2 (5,000 ppm of K-IBA) we obtained 21.50 in, for treatment 3 (10,000 ppm of K-IBA) 18.12 in for treatment 4 (10,000 ppm of Dip' N GrowTM) 17.32 in (Table 3.1). Dry weight for treatment 1 (control) 0.19 g, for treatment 2 (5000 ppm of K-IBA) we obtained 0.27 g, for treatment 3 (10,000 ppm of K-IBA) 0.25 g, for treatment 4 (10,000 ppm of Dip' N GrowTM) with 0.54 g had the greatest dry weight. For treatment 4 (Dip' N GrowTM) we could observe thicker roots and root length present compared to the root mass. This is presumably due to the treatment because Dip' N GrowTM is dissolved in alcohol and

maybe this influences directly the shoot length of the grey alder. Grey alder may be more susceptible to K-IBA hormone in comparison with other species that may grow normally.

According to Huss et al. (1980), *Alnus incana* cuttings consisted of internodes with leaf and auxiliary bud, and tip cuttings under controlled conditions. He concluded that cuttings consisting of one internode with the leaf and auxiliary bud attached rooted easily and more rapidly than shoot tip cuttings.

Hardwood cuttings:

For the three species used in this project red elm, grey alder, and buffaloberry cuttings, we did not observe any root formation, callus growth or shoot growth. There was no significant difference within and between treatments.

A study conducted by Schrader (2000) evaluated the time of collection and the use of indole-3-butyric acid (IBA) in the propagation of seaside alder [*Alnus maritima* (Marsh.)] by softwood cuttings. They used three different rates of IBA, (0,1, and 8 g×kg⁻¹) and concluded that the best rooted cuttings were the ones treated with higher amounts of IBA (8 g×kg⁻¹).

According to Boyer (2009), a study with two species of *Nyssa* spp. using two types of auxins: indole-3-butyric acid (IBA), and 1-naphthaleneacetic acid (NAA), with terminal and subterminal cuttings, concluded that almost 93% of the cuttings rooted when they had NAA compare to cuttings with IBA. Cuttings with NAA solutions produced 8 times more roots than those treated with IBA (Boyer, 2009).

The use of potassium salt indole-3-butyric acid (K-IBA) and the use of bottom heat made a positive effect while trying to root *Spigelia marilandica* cuttings (Goldberger, 2010). Cuttings were treated with 3000-6000 ppm of K-IBA and average rooting percentage was 76.6% while

cuttings with K-IBA have 46.9 percentage rooting. Cuttings that were placed in a heat pad from 81° F to 73° F increased rooting percentage from 48% to 85% (Goldberger 2010).

Conclusions

Red Elm and buffaloberry plants are difficult to propagate by cuttings, even when applying rooting hormones such as K-IBA and Dip 'N Grow™. Grey alder softwood cuttings are easy to propagate by cuttings when using low concentrations of K-IBA, and limited success was observed using Dip 'N Grow™ when larger shoot length is a desirable trait. Percentage rooting for treatment 2 (5000 ppm of K-IBA) was the most successful with a 96% .

Recommendations

Future projects should emphasize an individual species study using a variety of hormones at different concentrations. The reason for this is that each of the individual species has unique physiological characteristics which will allow them to respond differently to treatments in space and time.

Alternatives to Dip 'N Grow™ should be used along with K-IBA to determine if there is an improvement in shoot growth, and other desirable characteristics. Include semi-hardwood cuttings also and wood of different ages. A future project can focus on propagating these three species in vitro.

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Table 3.1 Grey Alder Cuttings

Treatments		Percentage rooting (%)	Root length (in)		Dry Weight (grams)
K-IBA (ppm)	Dip' N Grow™				
0		71	14.74	a ¹	0.19 b
5000		96	21.50	a	0.27 b
10000		79	18.12	a	0.25 b
	1:10	75	17.32	a	0.54 a

¹ lower case letters represent significant difference between treatments.